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The Supra- and Submolecular in Biology

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Current biochemistry is built on the molecular concept to which it owes most of its brilliant successes, such as the unravelling of intermediary metabolism, the isolation and chemical identification of hormones and vitamins, the crystallization of enzymes, and the establishment of the basic traits of proteins and nucleic acids, etc. In spite of these achievements, biochemistry has failed to bring us closer to the understanding of the more complex and subtle biological phenomena, like motion, nervous activity, secretion, or the establishment of concentration differences against a gradient, which all involve the transformation of chemical energy into some other form, mechanical, electric, or osmotic. These transformations are linked to the cellular structures which biochemists, interested in extracts, discard as the "residue". It is natural that biochemists should have worked first with what they could extract and bring into solution; and what is called "protein chemistry", today, should be mainly the chemistry of soluble proteins.

While the molecular concept has greatly helped to clarify the structure and function of extractable substances, it breaks down in the realm of biological structures, and its rigorous application actually retards progress.

One of the reasons that make the rigid molecular concept break down is that the electrons of one molecule may, under conditions, become located on the orbitals of another molecule, as shown by two closely related phenomena: charge transfer and conductivity. The first deals with the transfer of one electron between two isolated molecules, the latter with transfer in an assembly of a greater number of similar molecules. Both phenomena involve an overlap of orbitals which allows electrons to pass from the one to the other. Charge transfer may be symbolized by Fig. 1 in which the two sets of parallel lines on the left represent the highest filled and lowest empty orbitals of molecules D and A. If the electron clouds overlap and energy conditions are favorable, one of the two electrons of D, the donor, may pass to the empty level of A, the acceptor, the final situation being represented by Fig. 1 (b). In this state we actually have a

complex formed by two free radicals. The further reactions of the complex depend to a great extent on environmental conditions. The two molecules may remain linked together, the two electrons of D still coupled. In this case the optical spectrum of the complex may show similarities to that of the single radicals, but will give no electron spin resonance (ESR) signal. Under favorable conditions, the two molecules may part as free radicals, going into the ionic state. In this case, an ESR signal will be obtained. As

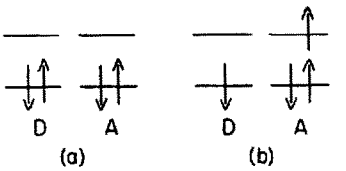

FIG. 1. Symbol of charge transfer.

has been shown earlier (Isenberg & Szent-Györgyi, 1958; Isenberg, Szent-Györgyi & Baird, 1960), biochemical pairs of biologically important substances, such as riboflavin and indoles, may form such complexes. Free radicals are most reactive but, even without going into the ionic state, the charge transfer complex D^+A^- may give unexpected reactions. The complex D^+A^- (Fig. 1 (b)), having an electron on a high lying orbital in A and an empty place on a low lying orbital in D, can be expected to be both a good electron acceptor and donor towards a third substance.

Semiconduction is schematically represented by Fig. 2 (a) and (b). In Fig. 2 (a) a number of similar molecules are brought into close proximity

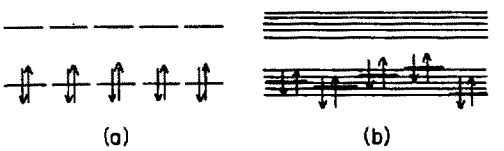

FIG. 2. Symbol of energy bands.

which allows the overlap of electronic clouds. In this situation the transition of an electron from one molecule to the other would be prevented by the Pauli principle which does not allow more than two electrons (of opposite spin) to have the same energy within the same system. But when the molecules are brought close together, they perturb one another, which changes the energy levels so that the real situation may be symbolized by Fig. 2 (b), in which the orbital energies have become slightly different. These single energy levels together form a package, a continuous band which, according to the number of participating units, may encompass a

great number of levels forming a quasi-continuous band. But, even in this case, no electrons could move in any preferred direction and the system could not conduct electricity, because all the ground levels are filled, containing two electrons of opposite spin, while the next highest energy band is empty. However, the system could be made conductant by taking away electrons from the ground level, creating "holes" in it, or by placing electrons on the empty level. Also, the system could become conductant if the empty level and filled level are close enough so that the energy of heat agitation is sufficient to raise an electron from the former to the latter, forming what is called a "semiconductor". The system would also be conductant if the single atoms forming the system had but one electron on their highest occupied level, as may be the case in metals.

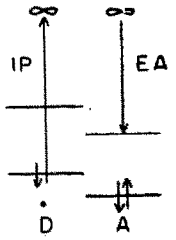

FIG. 3. Energy relations in charge transfer.

In an earlier paper I proposed that semiconduction might play a major part in energy transportation in living structures (Szent-Györgyi, 1941). The idea has not borne fruit because the lowest empty and highest filled bands are too far from one another, and the big energy quanta needed to raise an electron from the first to the latter are not available.

The way out of this situation is opened by the combination of the two reactions, charge transfer and the formation of energy bands. Let us suppose, for instance, that an electron is donated from the ground level of the system in Fig. 2 (b) to an extraneous molecule, leaving a "hole" behind. A hole having been created, the system would become electrically conductant. Similarly, if an extraneous donor would donate an electron to the empty band of Fig. 2 (b), this electron would have, here, a free mobility and could transport energy.

The energy change involved in a charge transfer reaction can conveniently be expressed by imagining the reaction taking place in two steps, in the first of which an electron is removed from D to infinity, while in the second, the electron is dropped from infinity to the empty orbital of A (Fig. 3). The energy needed to effect the first step is the ionization potential (IP) and is symbolized by the upward arrow in D of Fig. 3. The energy

gained in the second process is symbolized by the downward arrow in Fig. 3, and corresponds to the electron affinity (EA) of molecule A. The energy change would thus be $\Delta E = EA - IP + N$, N lumping up the various corrections to be made, due to interactions with the solvent, changes in coulombic forces, etc. If we use, in various charge transfer reactions different donors but always the same acceptor, then the energy change will depend mainly on the IP of the donors. The general final electron acceptor in higher biological systems is always O_2. This allows us to characterize the energy of any electron as its IP, or the "K-value" of its highest occupied orbital (Pullman & Pullman, 1952), which is a linear function of the IP. The lower the IP, or K, the less energy is needed for the transfer or the more energy will be gained by it. The lower the IP of the donor, or the higher the EA of the acceptor, the stronger the charge transfer. In a weak charge transfer only a small part of an electron is transferred, the two molecules remaining complexed and the two electrons of the original pair remaining coupled. Such complexes give no ESR signal. In a "strong" charge transfer a large part of one electric charge may be transferred, and the complex may dissociate into two free radicals which then give ESR signals.

If an aromatic structure with an extensive conjugated system interacts with another molecule of similar structure, the π pool of the first may donate an electron to the π pool of the latter in a so-called π–π interaction. This will be, for instance, the case in quinhydrone, where the π system of hydroquinone may transfer charge to the π system of the quinone.

Not only can conjugated systems act as donors. Atoms like O or N, having a "lone pair" of electrons, can donate one of their non-bonded electrons, acting as "local" electron donors.

The study of indole has led to the recognition of still another possibility. The indole derivatives interest the biologist because various biologically active substances, as growth hormones (indoleacetic acid), regulators (serotonin), or amino acids (tryptophan) belong to this group. It has been shown earlier that serotonin can form a strong charge transfer reaction with riboflavin. The result is contrary to expectation, since, with this relatively high K-value, indole cannot be expected to be a strong electron donor. If indole is made to react with one of the classical electron acceptors, like sym-trinitrobenzene (TNB), it actually behaves as a rather poor donor. However, when iodine is used as an acceptor, indole gives with it a black precipitate, which gives a very strong and narrow spin resonance signal, the g value of which is close to that of a free electron, indicating that a strong charge transfer has taken place (Szent-Györgyi, Isenberg & Baird, 1960). The I_2 molecule is not an especially strong acceptor. It is of about the same order as trinitrobenzene. The difference between the two

is that the I_2 molecule is relatively small, so it can also act as a "local" acceptor, interacting also with single C atoms or pairs of neighbouring C atoms. The calculations of the Pullmans (1952) show that the carbon in position 3 has an especially high electron density, while its neighbour, C_2, has but a somewhat lesser charge. These two atoms, conjugated with the whole π pool, are thus capable of acting as "local" donors, while drawing from the π pool for the electron to be donated.

As has been shown by the Pullmans (1955), carcinogenic hydrocarbons also have in their so-called "K-region" a pair of C atoms of high electron density, a pair which makes part of an extensive conjugated system.† It was found that the strongly carcinogenic hydrocarbons gave similar strong black charge transfer complexes with I_2, and the question occurred whether carcinogenesis is not connected with the ready donation of an electron in a "local" reaction. If so, then also carcinogens, which belong to other groups of substances and have no well-defined "K-region", should also give strong charge transfer complexes with I_2 which can be recognized by their black color. This was actually found to be the case. Hitherto, all strong carcinogens tested have given such a reaction (fluorenes, diphenyls, naphthylamines, azobenzenes), while their closely related non-carcinogenic homologues, or isomers, gave no such reaction.

The question arises—why, then, is indole not carcinogenic? All the carcinogens tested gave a strong charge transfer with TNB, as indicated by the dark red or purple color of their charge transfer complex, while indole gave with TNB only a weak charge transfer, as indicated by the faint yellow color of its charge transfer complex.‡ TNB indicates the tendency of the whole π pool to part with one of its electrons, as expressed, also, by the K-value of that molecule. Summing up, this experience suggests that a substance becomes carcinogenic if it is capable of giving off an electron in a "local" charge transfer, which is backed up by a strong donor tendency of a π pool. Why high polycyclic hydrocarbons like naphthacene, perylene, or violanthrene, which have a low K value and give strong charge transfer with I_2, are not carcinogenic is a different question. This may be due to their poor solubility or to strongly developed "L" regions, which, according to the Pullmans (1955) antagonizes carcinogenicity.

† The "K-region" should not be confused with the "K-values" discussed earlier in this paper. The K-values indicate the energy of the highest filled molecular orbital, while the K-region denotes two specific C atoms in aromatic hydrocarbons.

‡ The yellow color indicates that blue light was absorbed, that is, that the relatively high energy of blue light was needed to transfer an electron. A red or purple color means that light of relatively long wavelength is capable of transferring electrons and the curves of Fujimori (see Szent-Györgyi, 1960, p. 63) indicates that in this region spontaneous transfer becomes possible, that is, no light quanta are needed to transfer electrons.

This study of I_2 complexes shows that abstruse-looking quantum mechanical considerations may lead to interesting experiments bearing on important and urgent problems of current biochemistry. To spin this yarn farther, we might ask what happens to an electron donated to an empty energy band (Fig. 2 (b)). Such an empty band can, in all probability, be found in proteins with their H-bridges (Evans & Gergely, 1949; Cardew & Eley, 1959). In such a system the electron moves freely, belonging thus equally to the whole system and to all the molecules taking part in the building of that system. We do not know how extensive these systems are within the cells. Maybe, within the structural proteins, they extend over the whole cell, making one single electronic system of it, and so giving a deeper meaning to the cellular concept. The electron passing from a donor molecule to an acceptor molecule (Fig. 1 (b)) may also move on from the latter to the empty level of a third, from there to a fourth molecule, cascading down gradually to the level of O_2, the final biological electron acceptor, yielding gradually its energy, which then could be used by the cell for its function or maintenance. Such a fall, involving but one electron, does not entail any covalent changes. The underlying structure only forms, so to say, a quantum mechanical framework, in which these electronic changes can take place. So we arrive at biochemistry without chemistry if, by chemistry, we mean a rearrangement in molecular structures.

Possibly this subdivision of biochemistry into a chemistry of structures and soluble cellular components reflects a deeper regularity, however crude this division may be. As is generally known, the final source of all energy, driving life, is the radiation of the sun. The photon, interacting with matter on this globe, raises an electron to a higher energy level. As a rule the electron drops back to the ground level within a very short period, of the order of 10^{-8} secs. Life has learned to catch the electron in its excited state and utilize its excess energy. It seems likely that it was this excess energy of single electrons which in the beginning drove life and is still driving it today. The later developments chiefly concerned the storage and transport of this energy in the form of bond energy. Grossly speaking, the soluble cell constituents seem to be concerned, mainly, with this stabilization and *transportation* of chemical energy, while the structures which eventually use this energy, are concerned with the *transformation* of this bond-energy into the different sorts of work mentioned at the outset. My research work is led, at present, by the supposition that the bond-energy is reconverted eventually into the energy of single electrons when it has to interact with structure, maintain it in the "living state" (Szent-Györgyi, 1960), or produce those various sorts of work which underly the subtle and complex biological phenomena, such as motion, consciousness, thinking, etc. The function of soluble proteins can be duplicated *in vitro*

and expressed with symbols of classical chemical chemistry, letters and dashes, involving covalent changes, that is, changes in electron pairs, while the working of structures involves changes in distribution and energy levels of single electrons.†

Naturally, the building of protein structures involves covalent chemistry, involves the bonding of single molecules as the building of a house involves placing bricks side by side and linking them with mortar. But, once the house is finished, the bricks, as units, disappear and new "supramolecular" units, as walls, emerge, while the function of the wall is dominated by the "submolecular" qualities as thermic and mechanical properties of silicate particles of which bricks and mortar are made.

The duality of soluble and structure proteins may reflect not only the story of life, but also the story of biochemistry. When biochemistry went into bloom at the end of the last century the atom was an indivisible unit, molecules the aggregates of such units. Biochemistry was thus based on the rigid molecular concept. Later it was recognized that the atom is a whole universe and chemical reactions may be but the overall result of a series of subtler changes within these systems (e.g. polarization, intramolecular shift of electrons, etc.). This is broadening our outlook at present, leading to a better understanding of the mechanism of molecular reactions, as those of enzymes or coenzymes. The subtler and more complex biological phenomena, linked to structures, may necessitate a further extension of our outlook, a fusion of the molecular with the sub- and supra-molecular.

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† One may wonder how single electrons, with changes in their location, could induce those extensive changes in the physical state of living systems which go with function. This question can, at present, be approached only by speculation. However, it is not impossible that the principles of muscle activity represent a more general principle of biological function. We know from immune reactions, that the interaction of two specific proteins may induce profound changes in physical state (e.g. precipitin reactions). It has been shown in my earlier laboratory that muscle contraction comes about by the interaction of two proteins, actin and myosin. It has also been made probable that these two proteins, in resting muscle, are kept apart by repulsive forces. The repulsive forces are, in all probability, coulombic forces, decreasing with the second power of the distance, while the attractive forces are, to a great extent, van der Waals forces which vary with a much higher power of the distance. This must lead to a very subtle balance which can easily be disturbed, at least at one point by an electron, starting up a "zipper process".

T.B.

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